



Application of HR-LC-MS in screening methods for pesticides in cereals

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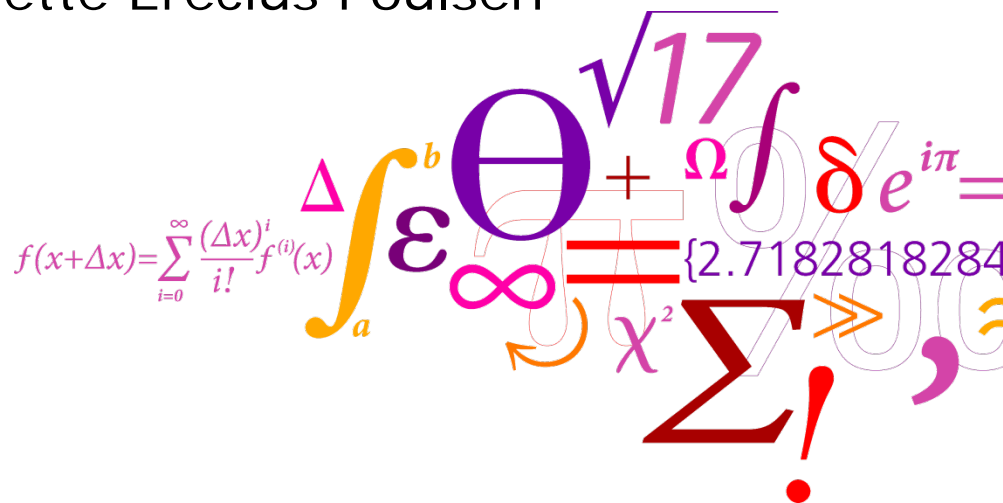
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Application of HR-LC-MS in screening methods for pesticides in cereals

Anne Kruse Lykkeberg, Mette Erecius Poulsen
Almeria, 12 June 2014



Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

Sample preparation of cereals – QuEChERS

LC-QTOF instruments



Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

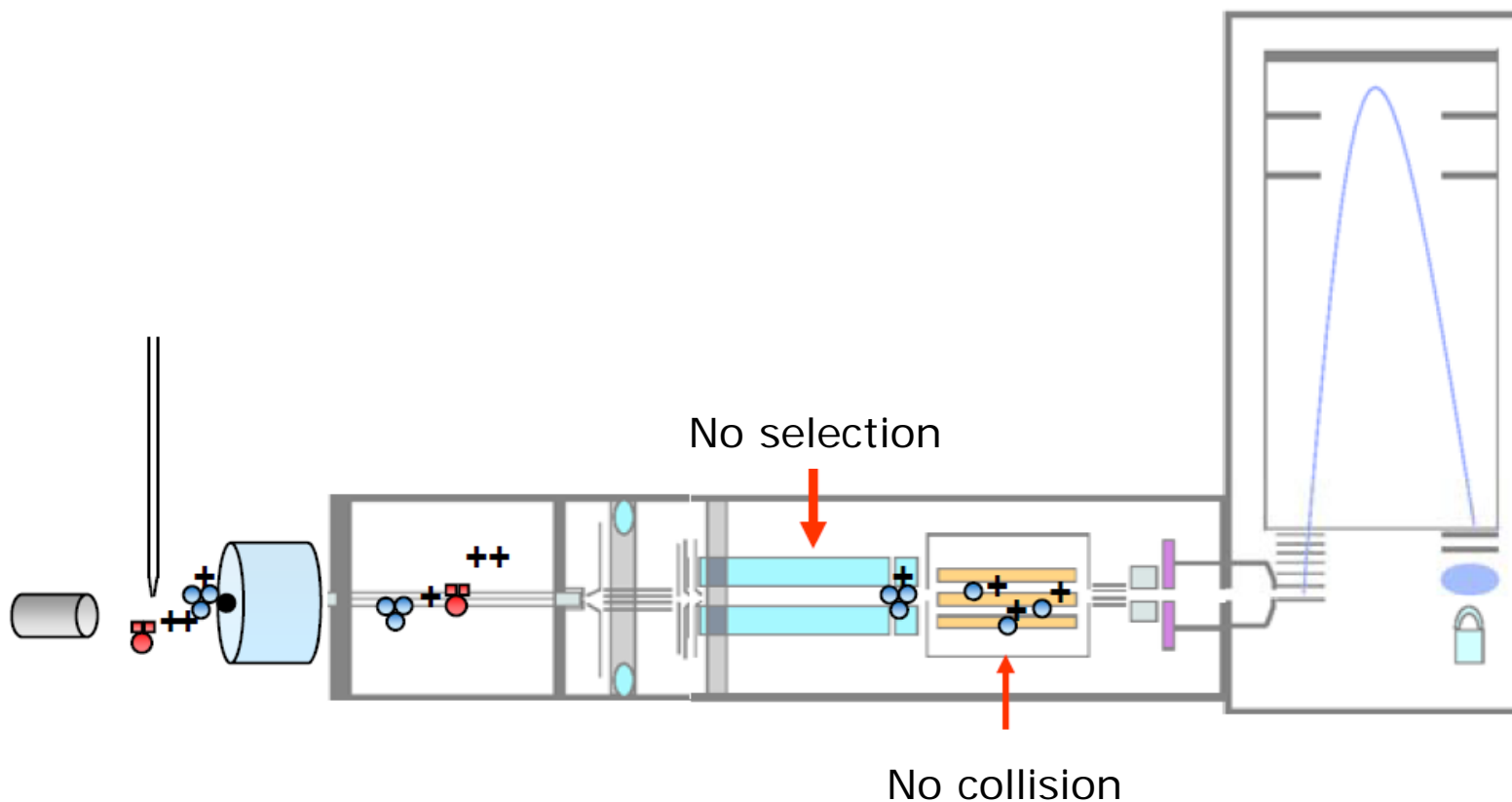
Sample preparation of cereals – QuEChERS

PCD – CSV file

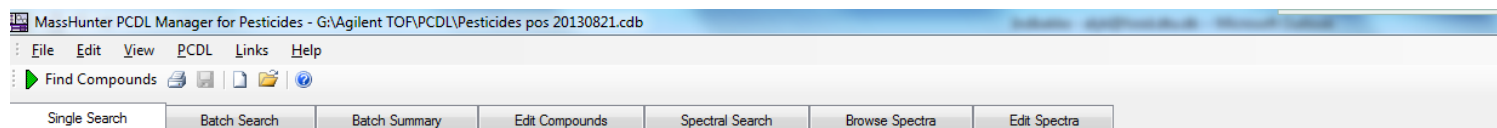
	A	B	C	D	E	F
22	# First line is 'Agilent TOF Formula data store'					
23	# Second line is 'Version:' followed by a version number					
24	#					
25	# Additionally	comments (such as these) may be inserted on individual				
26	# lines by specifying a '#' character at the beginning of the line					
27	#					
28	#					
29	### Formula	Retention Time	Mass	Compound name	Description	
30	# Formula	RT	Mass	Cpd	Comments	
31	C17H22O7	4.51	338.1365531	15 Acetyl DON		
32	C17H22O7		338.1365531	3 Acetyl DON		
33	C45H57N3O9	13	783.4094804	Beuvercin		
34	C19H26O7	5.46	366.1678532	DAS		
35	C15H20O6	3.47	296.1259884	Deoxynivalenol		
36	C36H63N3O9	13.83	681.4564306	Enniatin A		
37	C35H61N3O9	13.32	667.4407806	Enniatin A1		
38	C33H57N3O9	12.17	639.4094804	Enniatin B		
39	C34H59N3O9	12.75	653.4251305	Enniatin B1		
40	C34H59NO14	13.32	705.3935556	Fumonisin B2		

QTOF-analysis – MS scan

- Used to determine retention time



PCDL - library



Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
Acetazolamide	C4H6N4...	221.98813	<input type="checkbox"/>	<input type="checkbox"/>		59-66-5	1909	N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide	3
Acetochlor	C14ClNO...	269.11826	<input type="checkbox"/>	<input type="checkbox"/>	9.390	34256-82-1	1911	2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphe...	3
Acetyl-Seneciophylline	C20H25N...	375.16819	<input type="checkbox"/>	<input type="checkbox"/>		90341-45-0	4945366	11,16-Dioxo-13,19-didehydrosenecionan-12-yl ac...	0
Acibenzolar-S-methyl (CGA 245704)	C8N2OS2...	209.99215	<input type="checkbox"/>	<input type="checkbox"/>	8.490	135158-5...	77928	S-Methyl 1,2,3-benzothiadiazole-7-carbothioate	0
Acifluorfen	C14ClF3N...	360.99648	<input type="checkbox"/>	<input type="checkbox"/>	8.758	50594-66-6	40113	5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenz...	3
Acifluorfen-methyl	C15H9ClF...	375.01213	<input type="checkbox"/>	<input type="checkbox"/>		50594-67-7	82745	Methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nit...	0
Aclonifen	C12ClN2...	264.03017	<input type="checkbox"/>	<input type="checkbox"/>	9.906	74070-46-5	83411	2-Chloro-6-nitro-3-phenoxyaniline	4
Acrinathrin	C26F6NO...	541.13239	<input type="checkbox"/>	<input type="checkbox"/>	13.800	101007-0...	10469290	Cyano(3-phenoxyphenyl)methyl 3-[(1Z)-3-[(1,1,1,3...	0
ADBI (ABDI) (Celestolide)	C17H24O	244.18272	<input type="checkbox"/>	<input type="checkbox"/>		13171-00-1	55495	1-[1,1-Dimethyl-6-(2-methyl-2-propenyl)-2,3-dihydr...	0
Affinin	C14H23NO	221.17796	<input type="checkbox"/>	<input type="checkbox"/>		25394-57-4	4509783	(2E,6Z,8E)-N-Isobutyl-2,6,8-decatrienamide	0
AKH-7088	C19H16Cl...	476.05981	<input type="checkbox"/>	<input type="checkbox"/>		104459-8...	5005848	Methyl (((1E)-1-(5-[2-chloro-4-(trifluoromethyl)phe...	0
Acibenzolar-S-methyl (CGA 245704)	C8N2OS2...	209.99215	<input type="checkbox"/>	<input type="checkbox"/>	8.490	135158-5...	77928	S-Methyl 1,2,3-benzothiadiazole-7-carbothioate	0
Acifluorfen	C14ClF3N...	360.99648	<input type="checkbox"/>	<input type="checkbox"/>	8.758	50594-66-6	40113	5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenz...	3
Acifluorfen-methyl	C15H9ClF...	375.01213	<input type="checkbox"/>	<input type="checkbox"/>		50594-67-7	82745	Methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nit...	0
Aclonifen	C12ClN2...	264.03017	<input type="checkbox"/>	<input type="checkbox"/>	9.906	74070-46-5	83411	2-Chloro-6-nitro-3-phenoxyaniline	4
Acrinathrin	C26F6NO...	541.13239	<input type="checkbox"/>	<input type="checkbox"/>	13.800	101007-0...	10469290	Cyano(3-phenoxyphenyl)methyl 3-[(1Z)-3-[(1,1,1,3...	0
ADBI (ABDI) (Celestolide)	C17H24O	244.18272	<input type="checkbox"/>	<input type="checkbox"/>		13171-00-1	55495	1-[1,1-Dimethyl-6-(2-methyl-2-propenyl)-2,3-dihydr...	0
Affinin	C14H23NO	221.17796	<input type="checkbox"/>	<input type="checkbox"/>		25394-57-4	4509783	(2E,6Z,8E)-N-Isobutyl-2,6,8-decatrienamide	0
AKH-7088	C19H16Cl...	476.05981	<input type="checkbox"/>	<input type="checkbox"/>		104459-8...	5005848	Methyl (((1E)-1-(5-[2-chloro-4-(trifluoromethyl)phe...	0

PCDL – library - spectra

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search

Mass
Precursor ion:
Tolerance: 200 ppm mDa
Collision energy
Tolerance: 2.0 eV

Spectra for compound: Acetazolamide

Compound Name	Precursor Ion	Collision Energy
Acetazolamide	222.99541	
Acetazolamide	222.99541	

Graphic Mass List

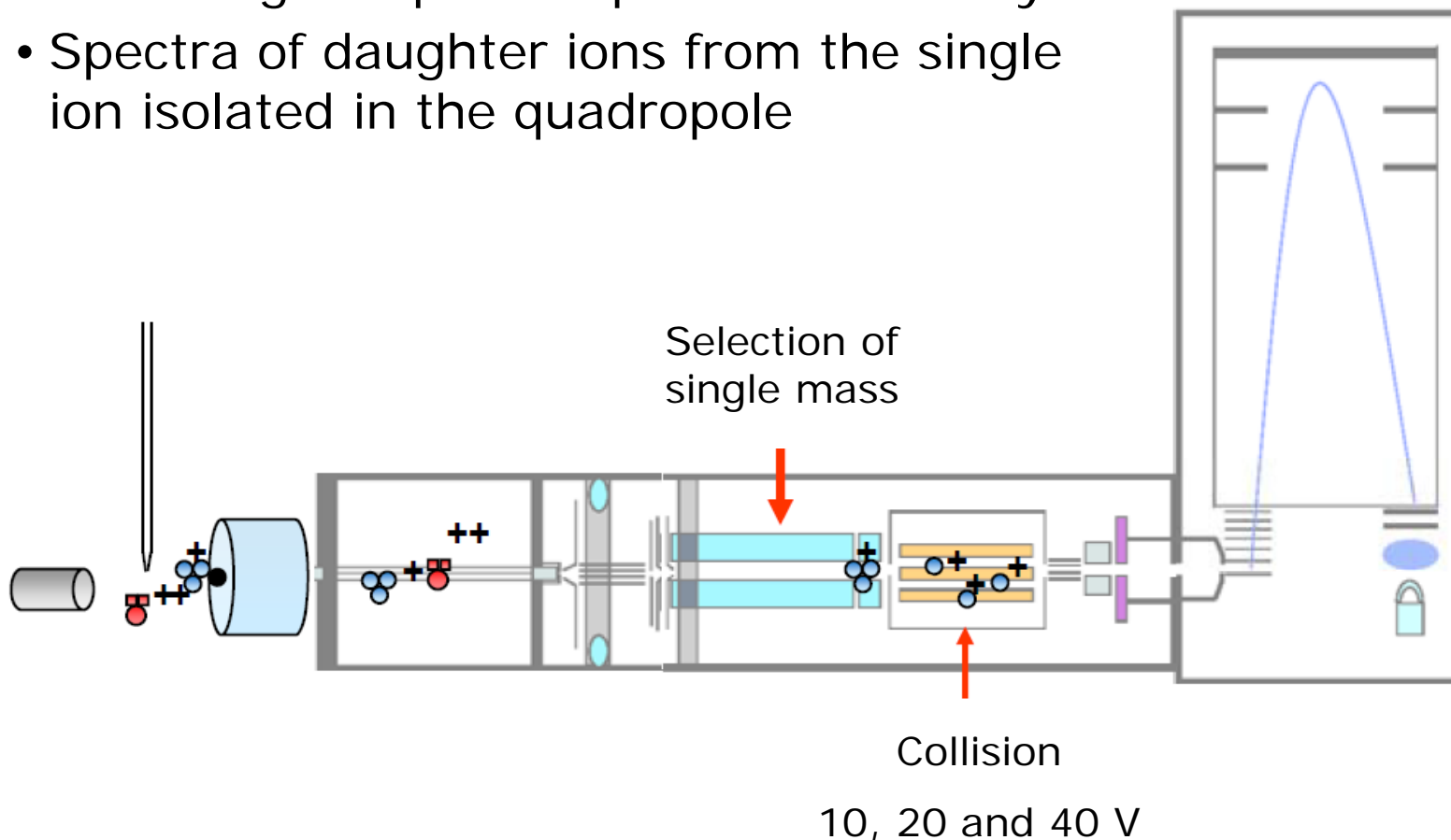
Mass	Rel Abund
180.98484	100.00000
222.99541	52.66977
163.95829	6.56664
205.96886	2.13168

Spectra for compound:

Compound Name	Precursor Ion	Collision Energy	Ion Polarity	Mode	Type
Acetazolamide	222.99541	10	Positive	ESI	QTOF
Acetazolamide	222.99541	20	Positive	ESI	QTOF
Acetazolamide	222.99541	40	Positive	ESI	QTOF

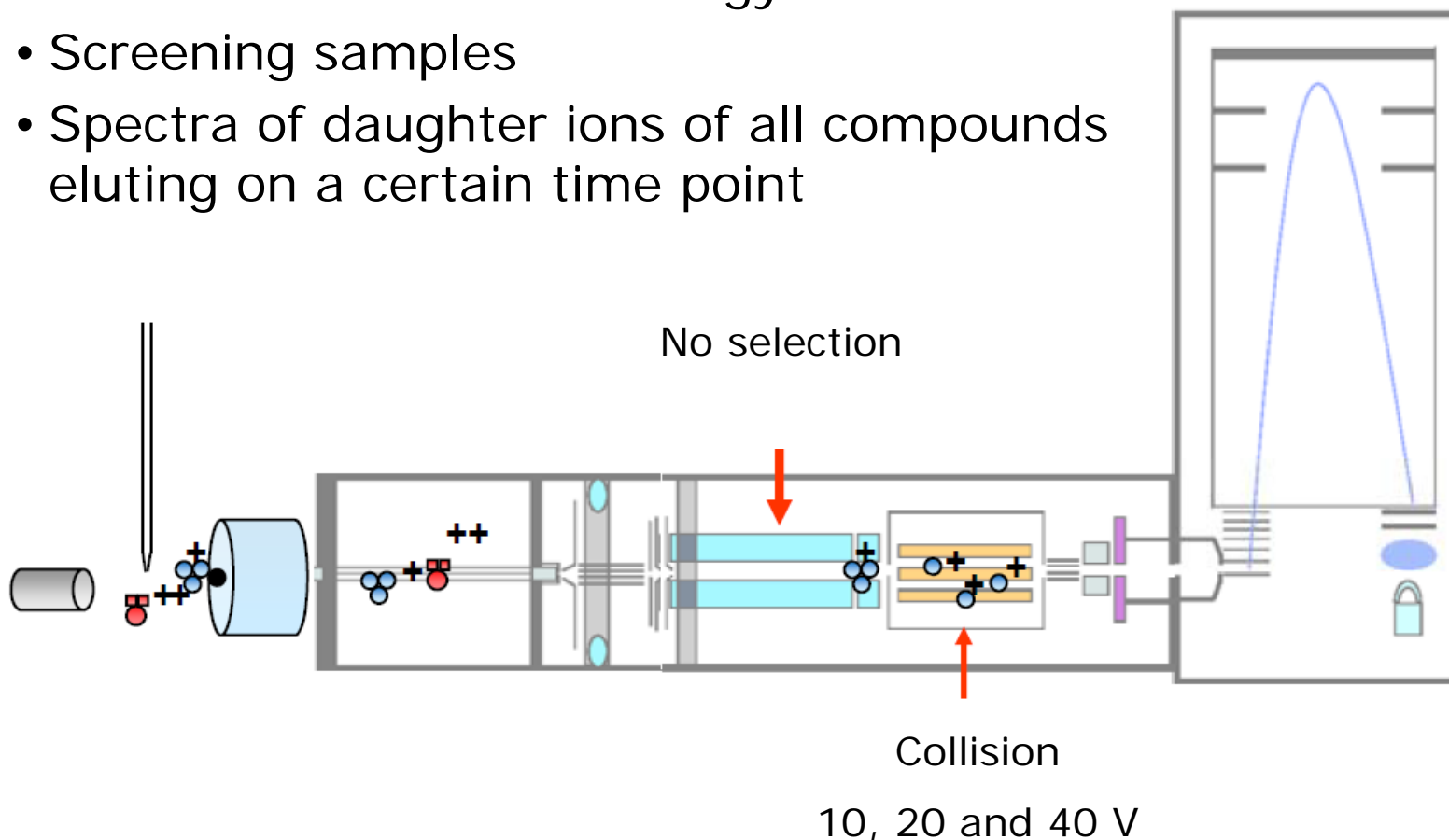
QTOF-analysis – Targeted MS/MS

- Obtaining compound spectra for Library
- Spectra of daughter ions from the single ion isolated in the quadropole

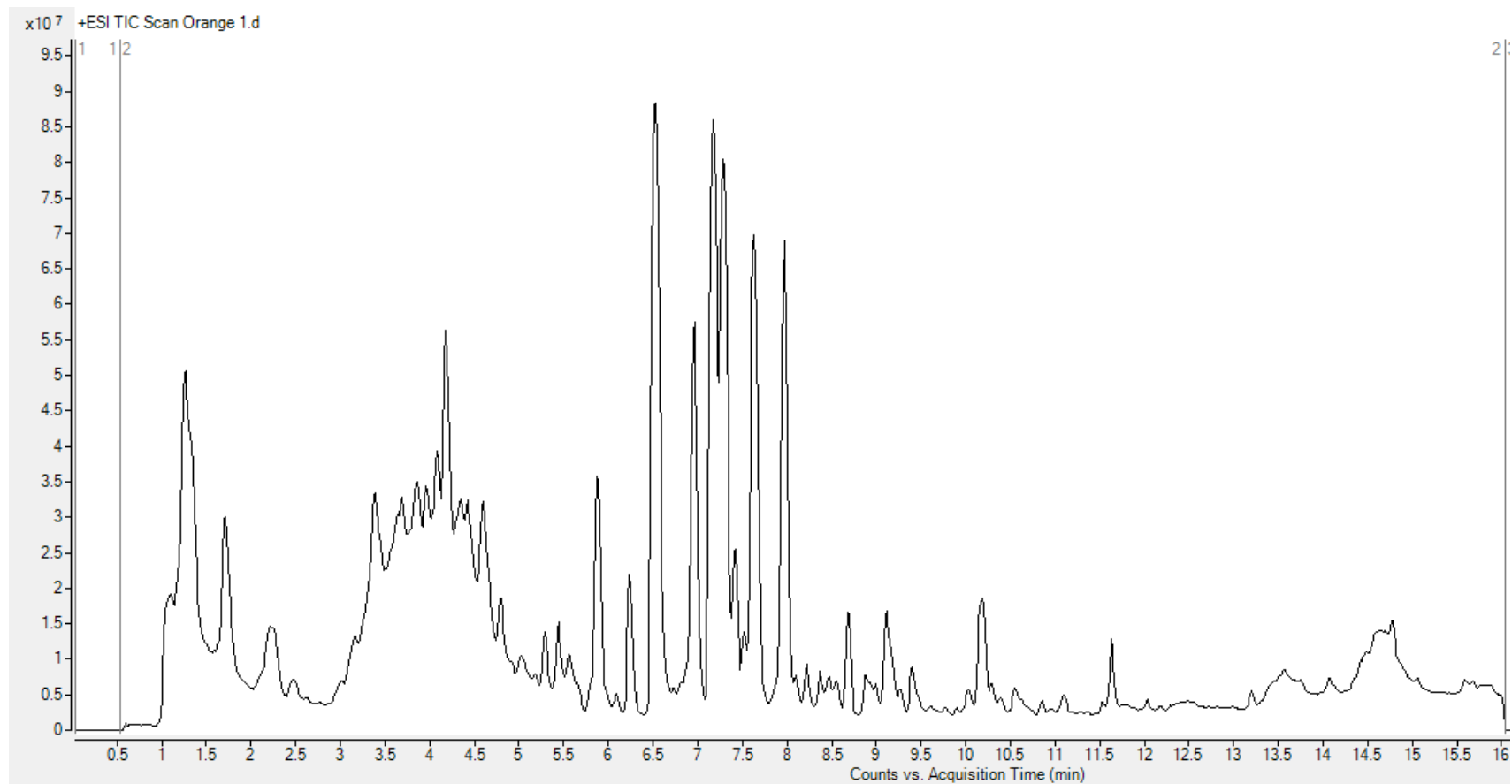


QTOF-analysis – All ion MS

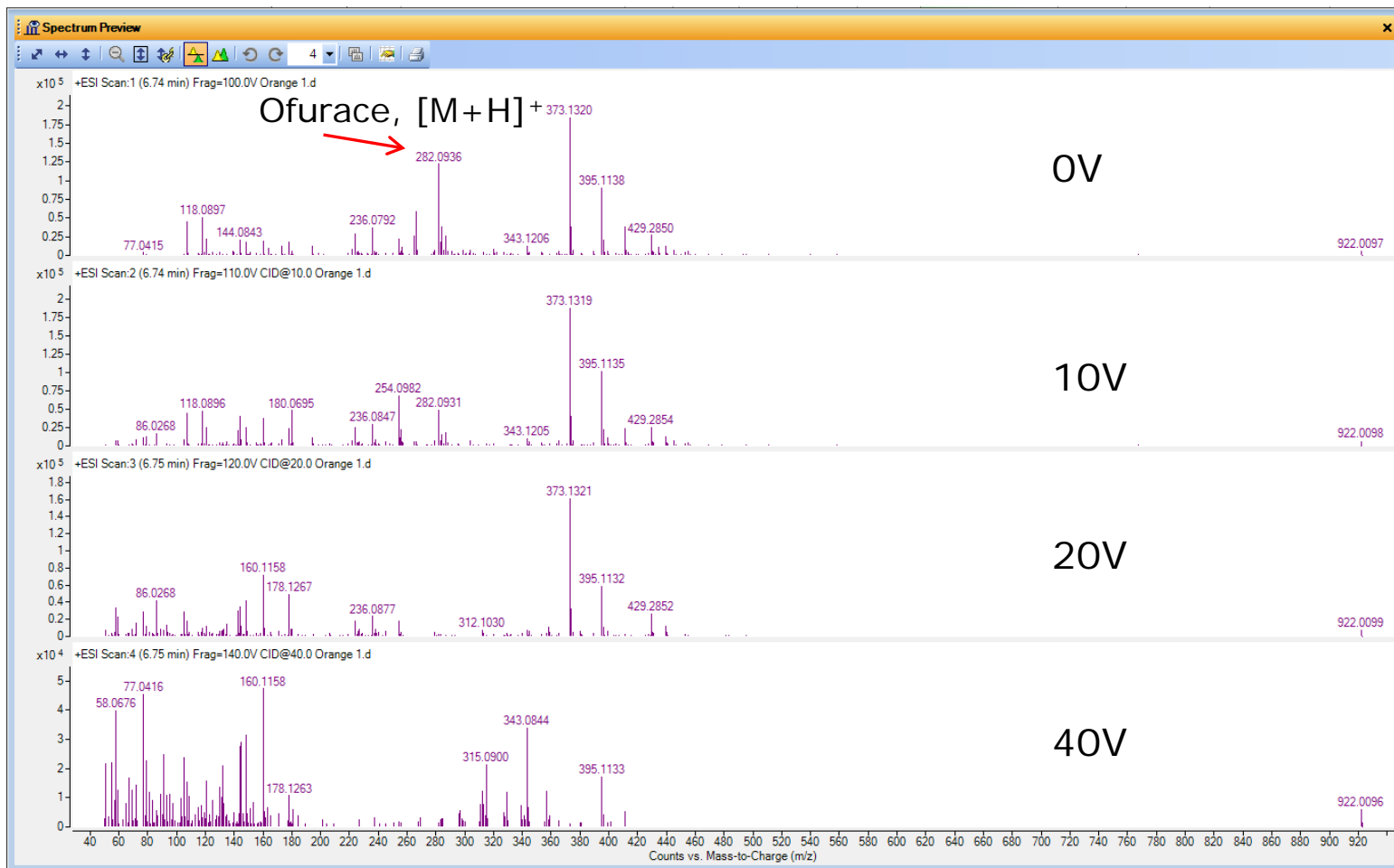
- MS scan with collision energy
- Screening samples
- Spectra of daughter ions of all compounds eluting on a certain time point



Chromatogram - TIC



Spectra – MSscan incl. collision



Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

Sample preparation of cereals – QuEChERS

Standard mixtures

Mixtures

50-100 pesticides

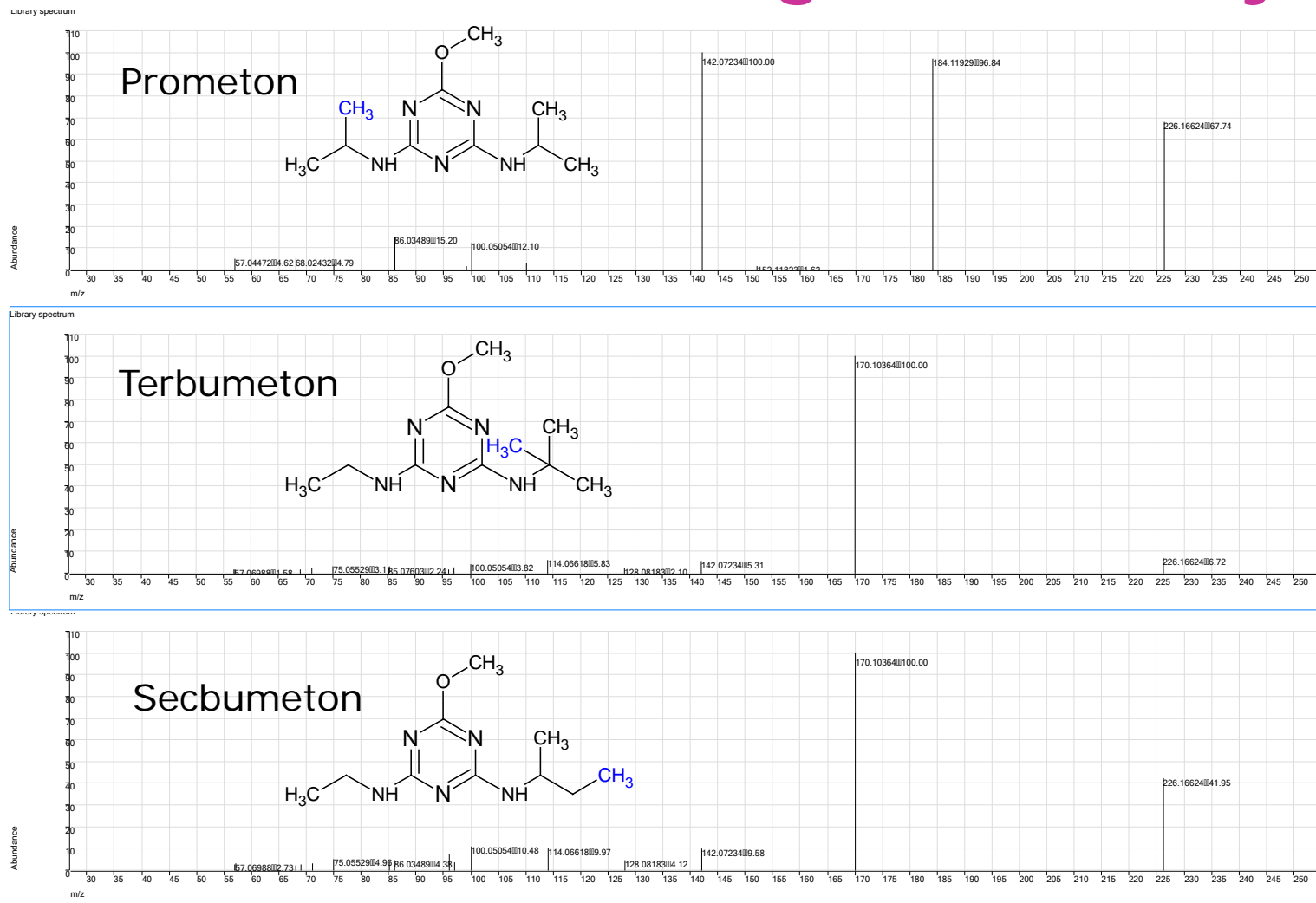
Isomers

Avoiding isomers in the mixtures

Validation

2-6 mixtures together

Spectra of isomers in Agilents library



Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

Sample preparation of cereals – QuEChERS

Validation

- My alternative validation plan
- 20-25 samples – spiked at 0.1 mg/kg
- 10-20 blank samples

	Spiked – 0.1 mg/kg	Blank
Rice	5	5
Oat	5	5
Barley	5	5
Wheat	5	5
Rye	5	5

Dilution of spiked samples

Rye	0.1 mg/kg	Blank
0.10 mg/kg	1000 µl	-
0.02 mg/kg	200 µl	800 µl
0.01 mg/kg	100 µl	900 µl
Blank	-	1000 µl

Spiked rye is diluted with Blank rye ...

Matrix is the same

Recovery is assumed to be the same at the different concentration levels

Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

Sample preparation of cereals – QuEChERS

Compound list – exported to Excel

Name	File	Area	Flags (Tgt)	Score (Tgt)	RT Diff (Tgt)	Diff (Tgt, ppm)	RT	Fls Co	Label
Aldicarb	Pos Byg 0_1-1.d	137207	No H adduct;Qualified	92.1	-0.059	-1.46	5.337	80	Cpd 11: Aldicarb
Aldicarb-sulfone (Aldoxycarb)	Pos Byg 0_1-1.d	1459914	low score;Not qualified	73.93	-0.05	-2.92	4.681	0	Cpd 36: Aldicarb-sulfone (Aldoxy
Aldicarb-sulfoxide	Pos Byg 0_1-1.d	17148	multiple IDs;Not qualified	75.48	0.009	1.08	3.229	0	Cpd 18: Aldicarb-sulf
Amidosulfuron	Pos Byg 0_1-1.d	299303	Qualified	77.56	-0.122	-0.8	6.273	80	Cpd 194: Amidosul
Aminocarb	Pos Byg 0_1-1.d	88888	Qualified	82.26	-0.036	1.77	1.484	20	Cpd 22: Amini
Amitraz	Pos Byg 0_1-1.d	187007	low score;Qualified	64.64	0.291	-2.59	13.031	60	Cpd 98: An
Anthraquinone	Pos Byg 0_1-1.d	182161	low score; No H adduct;Not qualified	63.47	0.127	4.02	7.837	0	Cpd 21: Anthraqu
Atrazine	Pos Byg 0_1-1.d	2655497	Qualified	89.22	-0.072	-0.33	6.608	100	Cpd 29: Atr
Azaconazole(II)	Pos Byg 0_1-1.d	63278	low score; No H adduct;Not qualified	35.65	-0.203	-8.81	6.441	0	Cpd 105: Azaconaz
Azimsulfuron (IN A8947)	Pos Byg 0_1-1.d	144789	Qualified	89.01	-0.068	-1.24	6.51	40	Cpd 228: Azimsulfuron (IN Ai
Azinphos-ethyl (Guthion ethyl)	Pos Byg 0_1-1.d	120726	Qualified	75.62	-0.127	-0.64	9.373	60	Cpd 171: Azinphos-ethyl (Guthion
Azoxystrobin	Pos Byg 0_1-1.d	3200956	Qualified	83.17	-0.098	0.72	8.228	100	Cpd 219: Azoxyst
Benalaxyl	Pos Byg 0_1-1.d	3780302	low score;Qualified	74.98	-0.141	0.79	9.819	100	Cpd 146: Benz
Bendiocarb	Pos Byg 0_1-1.d	280916	Qualified	84.75	-0.083	1.21	6.147	100	Cpd 38: Bendi
Benfuracarb	Pos Byg 0_1-1.d	272873	Qualified	80.34	-0.107	-0.43	11.663	60	Cpd 224: Benfur
Bensulfuron-methyl	Pos Byg 0_1-1.d	658684	Qualified	94.14	-0.049	-0.44	7.111	60	Cpd 222: Bensulfuron-n

Export

Export type

File type: Data as Excel file (*.xls)

With delimiter: .

Export contents

☐ Only highlighted rows
 ☒ All rows

Export destination

☒ Auto-generate a file at data file location
 ☐ Specified file:

OK

Cancel

All file gathered in one Excel-file

	N	O	P	Q	R	S	T	U	V	AW	AX	AY	AZ	BA	BB	BC	BD	BE	
1																			
2																			
3	Pred M	Sequer	Seq Na	Seq Typ	Tgt Seq	AGILEN	CAS	Compo	Name	Score D	Abund	Algorit	Area	Avg Ma	Base Pe	Z Count	CE	MS/MS	File
4							30560-19-1		Acephate(I)		19138	Find By Fc	153900		189.1598	1			Pos
5							135410-20-7		Acetamiprid		114050	Find By Fc	1385340		190.0434	1			Pos
6							50594-66-6		Acifluorfen		18863	Find By Fc	82018		121.0508	1			Pos
7							2701-86-2		Akton oxon		67015	Find By Fc	1147525		121.0509	1			Pos
8							116-06-3		Aldicarb		15656	Find By Fc	115981		121.0509	1			Pos
9							1646-88-4		Aldicarb-sulfone (Al		114050	Find By Fc	1055620		190.0434	1			Pos
10							120923-37-7		Amidosulfuron		12554	Find By Fc	314108		304.264	1			Pos
11							33089-61-1		Amitraz		120052	Find By Fc	758480		121.0507	1			Pos
12							1912-24-9		Atrazine		243062	Find By Fc	2260854		207.1496	1			Pos
13							1007-28-9		Atrazine-desisoprop		3631	Find By Fc	32746		207.1496	1			Pos
14							120162-55-2		Azimsulfuron (IN A8		5492	Find By Fc	121926		121.0508	1			Pos
15							2642-71-9		Azinphos-ethyl (Gut		21377	Find By Fc	176158		121.0509	1			Pos
16							131860-33-8		Azoxystrobin		55627	Find By Fc	2119022		404.1245	1			Pos
17							71626-11-4		Benalaxy		215368	Find By Fc	3107846		121.0508	1			Pos
18							22781-23-3		Bendiocarb		27395	Find By Fc	204079		274.2537	1			Pos
19							82560-54-1		Benfuracarb		263928	Find By Fc	2615067		356.2437	1			Pos
20							83055-99-6		Bensulfuron-methyl		30913	Find By Fc	421688		121.0509	1			Pos
21							55179-31-2		Bitertanol		10747	Find By Fc	65937		224.1193	1			Pos
22							188425-85-6		Boscalid (Nicobifen)		10513	Find By Fc	110547		121.0509	1			Pos
23							116255-48-2		Bromuconazole(I)		23265	Find By Fc	289466		121.0508	1			Pos
24							116255-48-2		Bromuconazole(II)		16231	Find By Fc	209939		121.0509	1			Pos
25							1563-66-2		Carbofuran		9774	Find By Fc	1257818		274.2533	1			Pos
26							16655-82-6		Carbofuran, - 3 hydro		34797	Find By Fc	921386		203.0928	1			Pos
27							55285-14-8		Carbosulfan		235551	Find By Fc	1847735		680.5103	1			Pos
28							5234-68-4		Carboxin		159397	Find By Fc	1144864		121.0509	1			Pos
29							143807-66-3		Chromafenozide		90344	Find By Fc	919310		226.1346	1			Pos
30							94593-91-6		Cinosulfuron		38738	Find By Fc	435723		121.0509	1			Pos
31							99129-21-2		Clethodim(I)		5171	Find By Fc	51484		121.0508	1			Pos
32							105512-06-9		Clodinafop-propargyl		45525	Find By Fc	330422		121.0508	1			Pos

Area of all samples spiked at 10 ppb

	A	B	C	D	E	F	G	H	I
1	Skabelon: jhia 27/2-2013 (NB: Celler er ikke låste, så ændringer skal kontrolleres)								
2									
3	Kolonne med stofnavn	V	V	V	V	V	V	V	V
4	Kolonne med data	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ
5	Nedeste række med data	200	200	200	200	200	200	200	200
6									
7	Data	Area	Area	Area	Area	Area	Area	Area	Area
8	Faneblad	Hvede 0_01-1	Hvede 0_01-2	de 0_01-3	de 0_01-4	Hvede 0_01-5	yg 0_01-1	yg 0_01-2	yg 0_01-3
53	Chlormephos	-----	-----	-----	-----	-----	-----	-----	-----
54	Chlorobenzilate	-----	-----	-----	-----	-----	-----	-----	-----
55	Chlorpropham	-----	-----	-----	-----	-----	-----	-----	-----
56	Chlorothalonil	-----	-----	-----	-----	-----	-----	-----	-----
57	Chlorpropylate	-----	-----	-----	-----	-----	-----	-----	-----
58	Chlorpyrifos	-----	-----	-----	-----	-----	-----	-----	-----
59	Chlorpyrifos-methyl	-----	-----	-----	-----	-----	-----	-----	-----
60	Chlorthal-dimethyl	-----	-----	-----	-----	-----	-----	-----	-----
61	Chromafenozide	141344	142148	112840	122602	140363	132370	138340	142966
62	Cinidon-ethyl	-----	-----	-----	-----	-----	-----	-----	-----
63	Cinosulfuron	88804	75673	59014	67722	74279	69385	63358	65773
64	Clethodim(II)	-----	-----	-----	-----	-----	-----	-----	-----
65	Clodinafop-propargyl	40158	30783	40762	-----	36048	37315	33719	78682
66	Clofentezin	-----	-----	-----	-----	-----	-----	-----	-----
67	Clomazone	52924	49092	52854	46925	52430	43874	45868	53189
68	Clothianidin	-----	-----	-----	-----	-----	-----	-----	-----

Comparison of areas in spiked samples with blank

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Skabelon: jhia 27/2-2013 (NB: Celler er ikke låste, så ændringer skal kontrolleres)												
2													
3	Kolonne med stofnavn	A	A	A	A	A	A	A	A	A	A	A	A
4	Kolonne med data	Y	Y	Y	X	AA	AA	AA	Y	AA	AA	AA	Y
5	Nedeste række med data	400	400	400	400	400	400	400	400	400	400	400	400
6		MIN	MIN	MIN	Max								
7	Data	Areal	Min	Min	Max	N	N	N	N	Med mindst et fragment			
8	Faneblad	pos0.01	pos0.02	pos0.1	pos blank	pos0.01	pos0.02	pos0.1	pos blank	ment 0.01	ment 0.02	ment 0.1	ment blank
201	Mesotrione	0	0	0	0	0	0	0	0	0	0	0	0
202	Metaflumizone	0	0	0	0	0	0	0	0	0	0	0	0
203	Metalaxyl(I)	315804	724716	231227	64922	20	20	17	2	20	18	14	0
204	Metalaxyl(II)	0	58865	81263	0	0	1	15	0	0	0	0	0
205	Metamitron	164622	329042	92575	0	19	18	15	0	16	14	12	0
206	Metconazole	75837	122612	113384	80481	20	20	20	3	0	0	4	0
207	Methacrifos	262153	494818	2276131	0	1	3	3	0	0	0	0	0
208	Methamidophos(I)	0	0	0	0	0	0	0	0	0	0	0	0
209	Methamidophos(II) (Metamidopho	0	0	37388	0	0	0	10	0	0	0	3	0
210	Methidathion	0	36634	167709	0	0	20	17	0	0	18	15	0
211	Methiocarb (Mercaptodimethur)	0	31632	42811	0	0	11	7	0	0	0	0	0
212	Methiocarb sulfone	32456	66421	383397	0	13	13	13	0	0	0	0	0
213	Methiocarb sulfoxide	89617	487522	101424	0	19	17	15	0	12	12	10	0
214	Methomyl	0	32143	31662	0	0	1	7	0	0	0	5	0
215	Methoprene	0	0	0	0	0	0	0	0	0	0	0	0
216	Methoxychlor	0	0	0	0	0	0	0	0	0	0	0	0

Disposition

Analysis at the instrument

Standard mixtures

Validation plan

Validation

Virtual computers – for data treatment

Sample preparation of cereals – QuEChERS

Virtual computers for data-analysis

Challenges

- The Agilent software demands Windows Professional 64 bit
 - english version
- Back up of all laboratory computer on LAB-domain
- Laboratory domain, with no access from personal computers

Solution

- Virtual computers from Citrix
 - On the Laboratory domain
 - Windows Professional 64 bit
 - Access to all data – back up early in the morning
 - Few licenses are necessary
 - Access from all computers with internet

Disposition

Analysis at the instrument

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Sample preparation of cereals – QuEChERS

FVII cocktail IV – project

- Aim is to develop a cereal-method to analyze many analytes in one method.
- Analytes:
 - Pesticides (The pesticides most present in cereal – in practice the standard mixtures which we use for our quantitative methods).
 - Mycotoxins: Most important mycotoxins in cereals.

Method development - QuEChERS

Extraction

- Water
- Acetonitrile

Phase separation

- Magnesium sulphate
- NaCl, Citrate buffer

Freeze out step

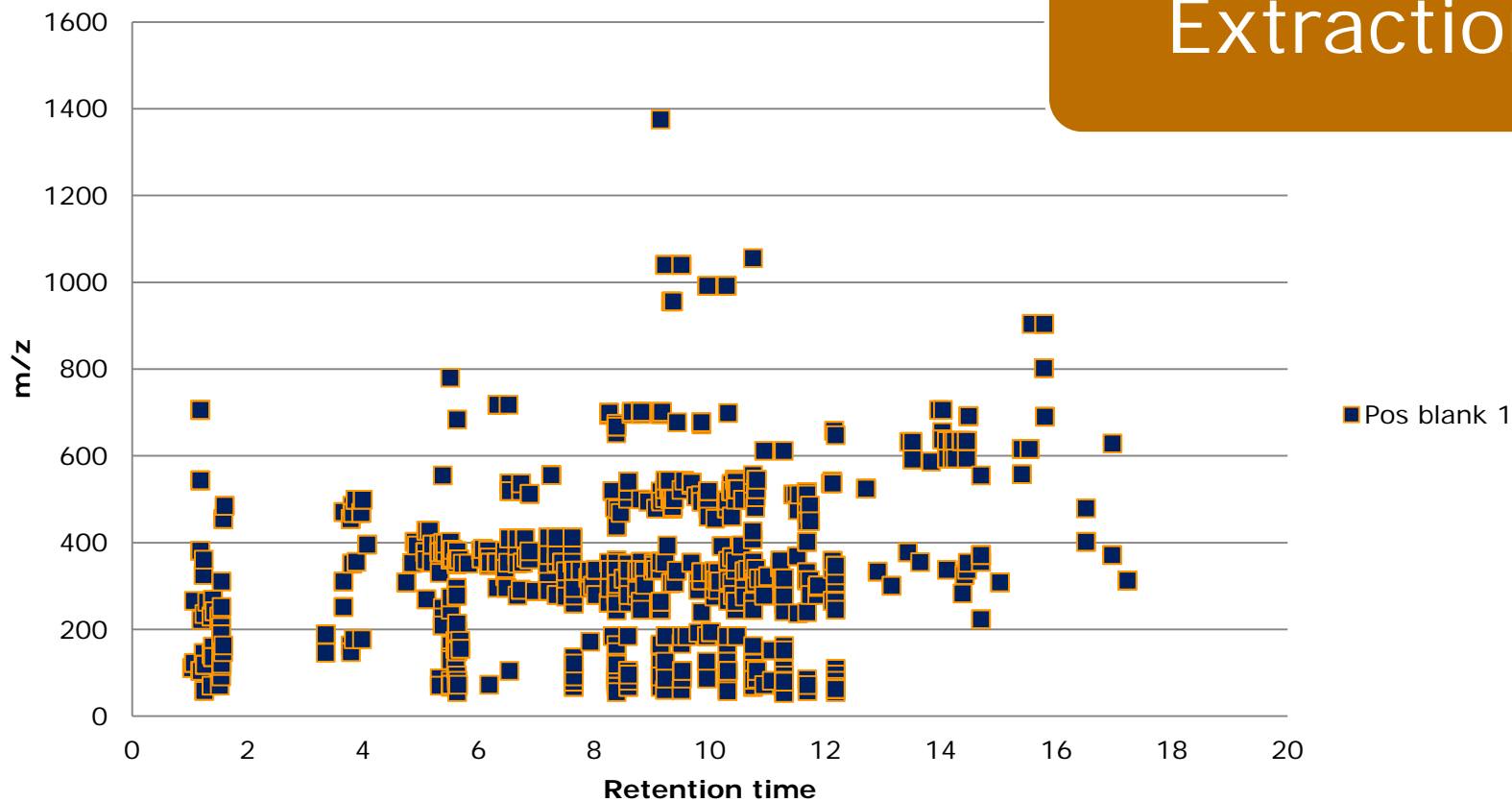
- 1 h at -80 °C
- Centrifugation

PSA clean up

- PSA
- Magnesium sulphate

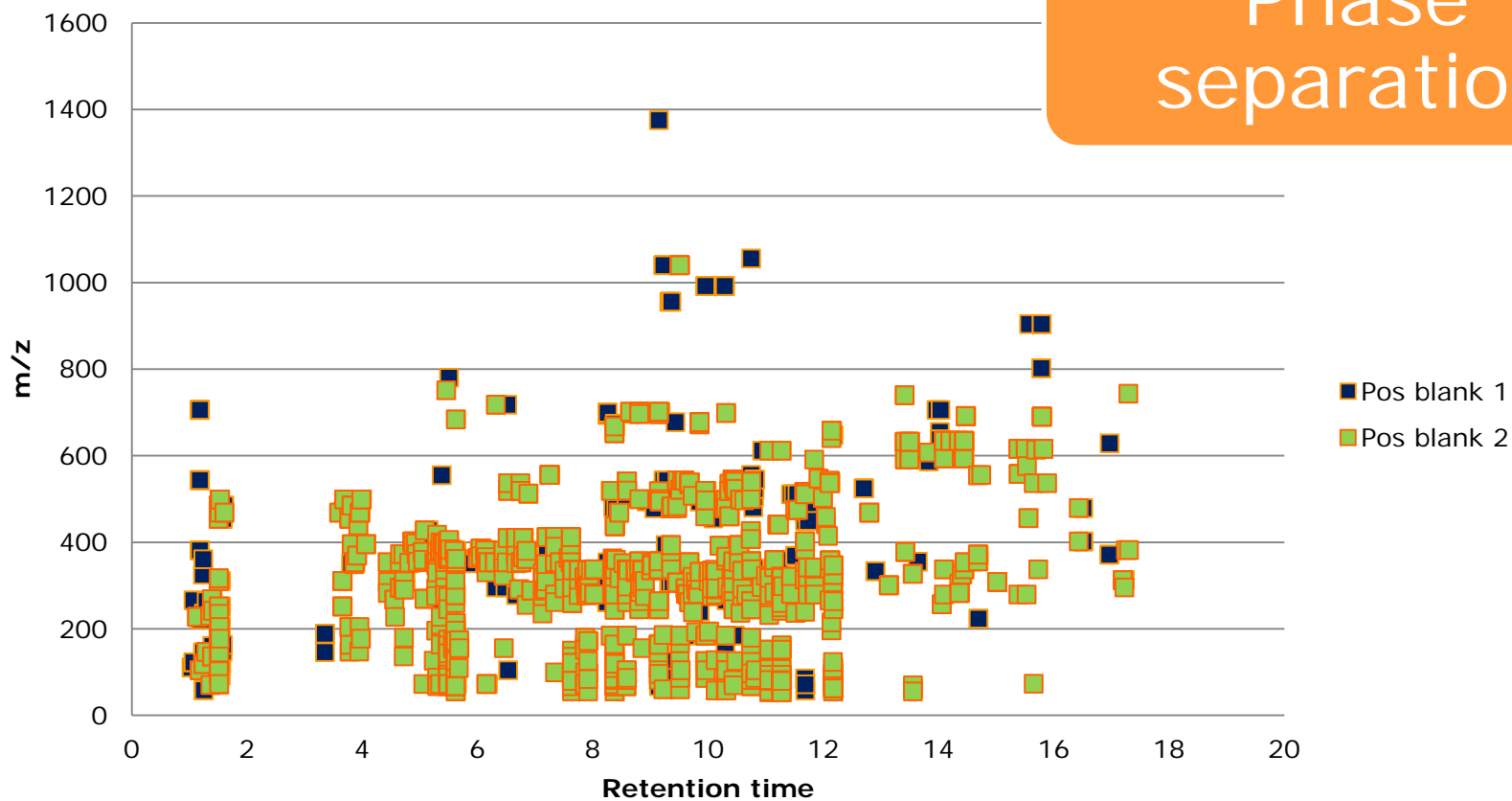
Positive mode

Extraction



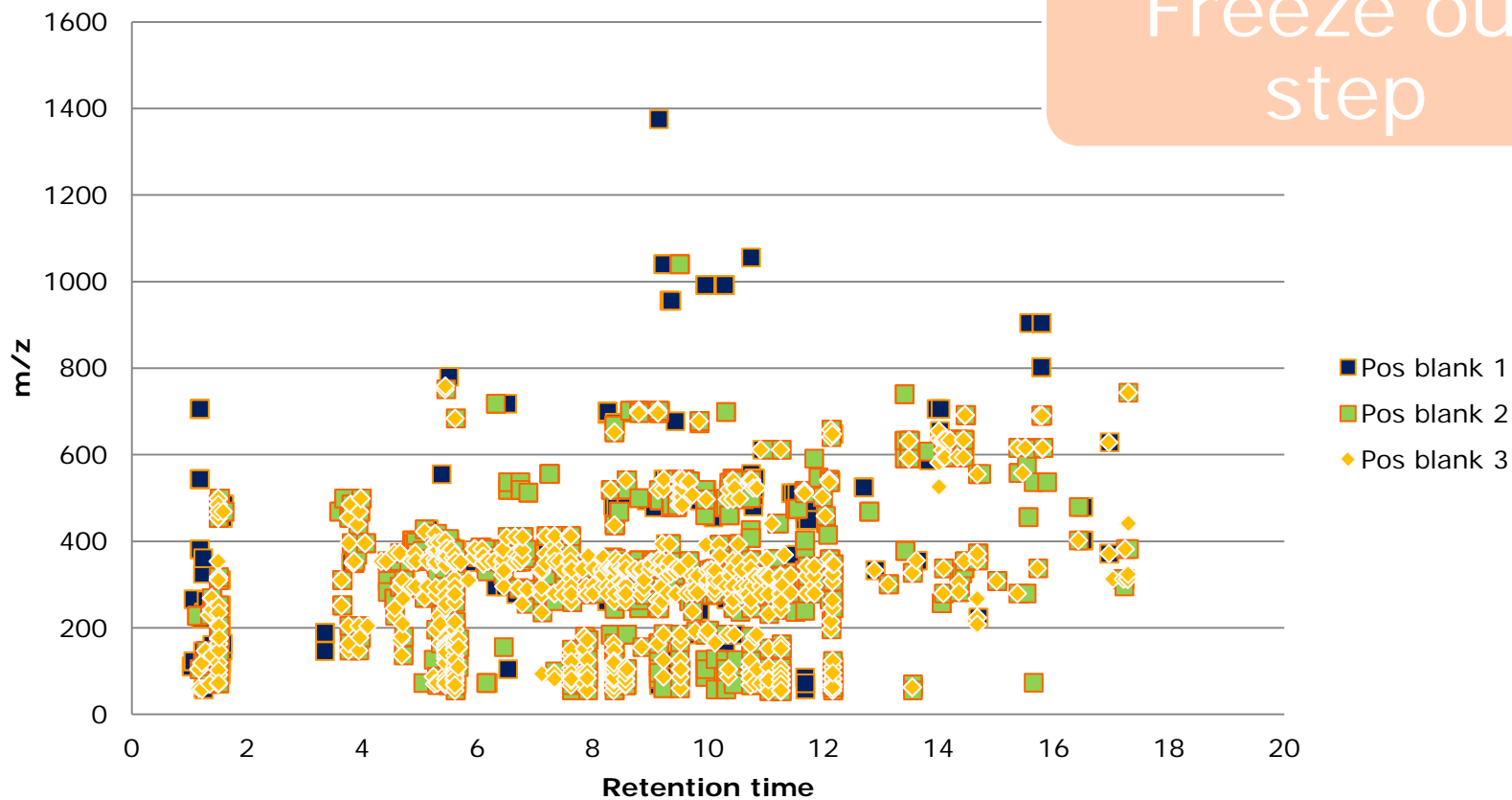
Positive mode

Phase
separation



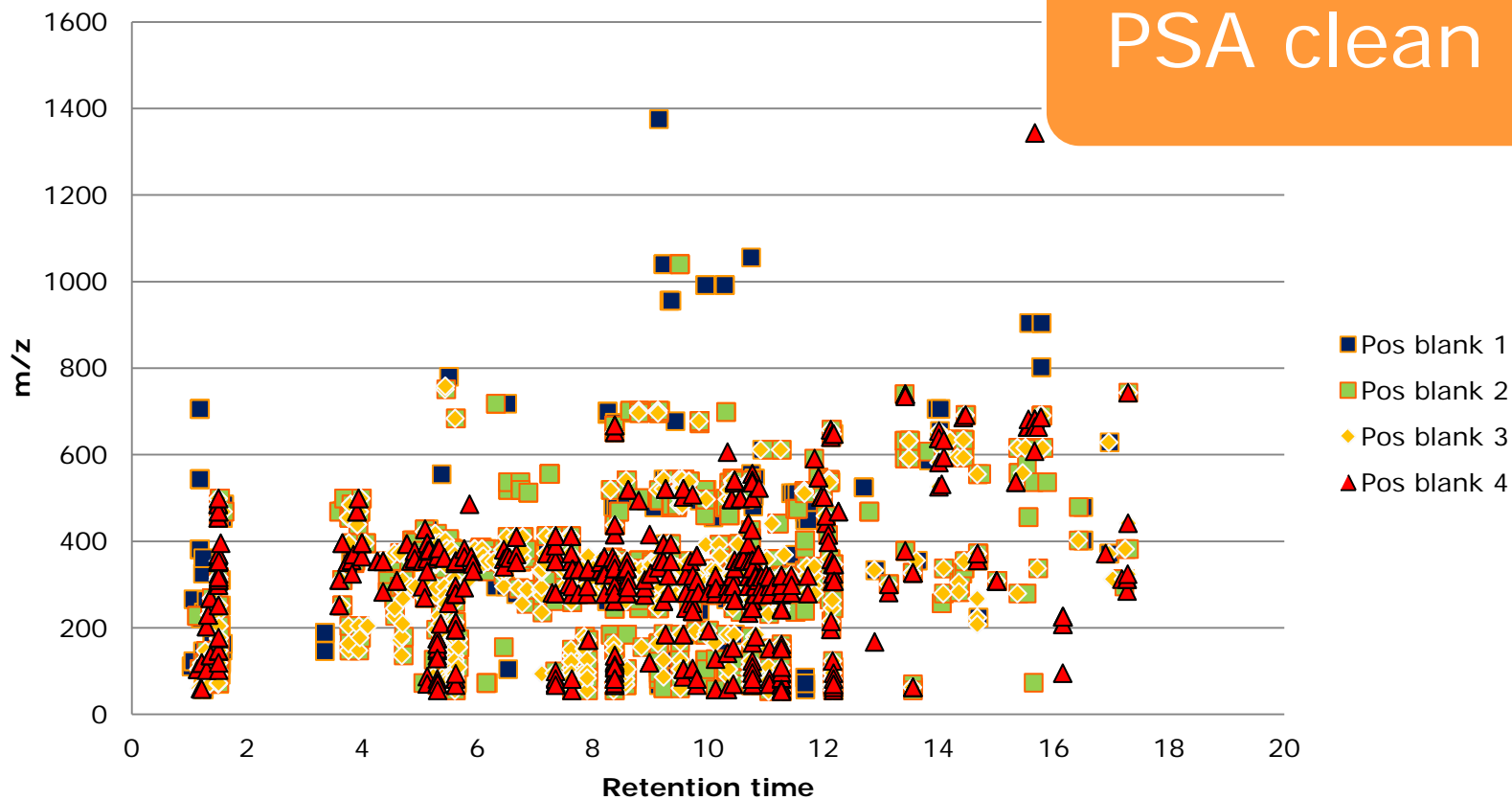
Positive mode

Freeze out
step



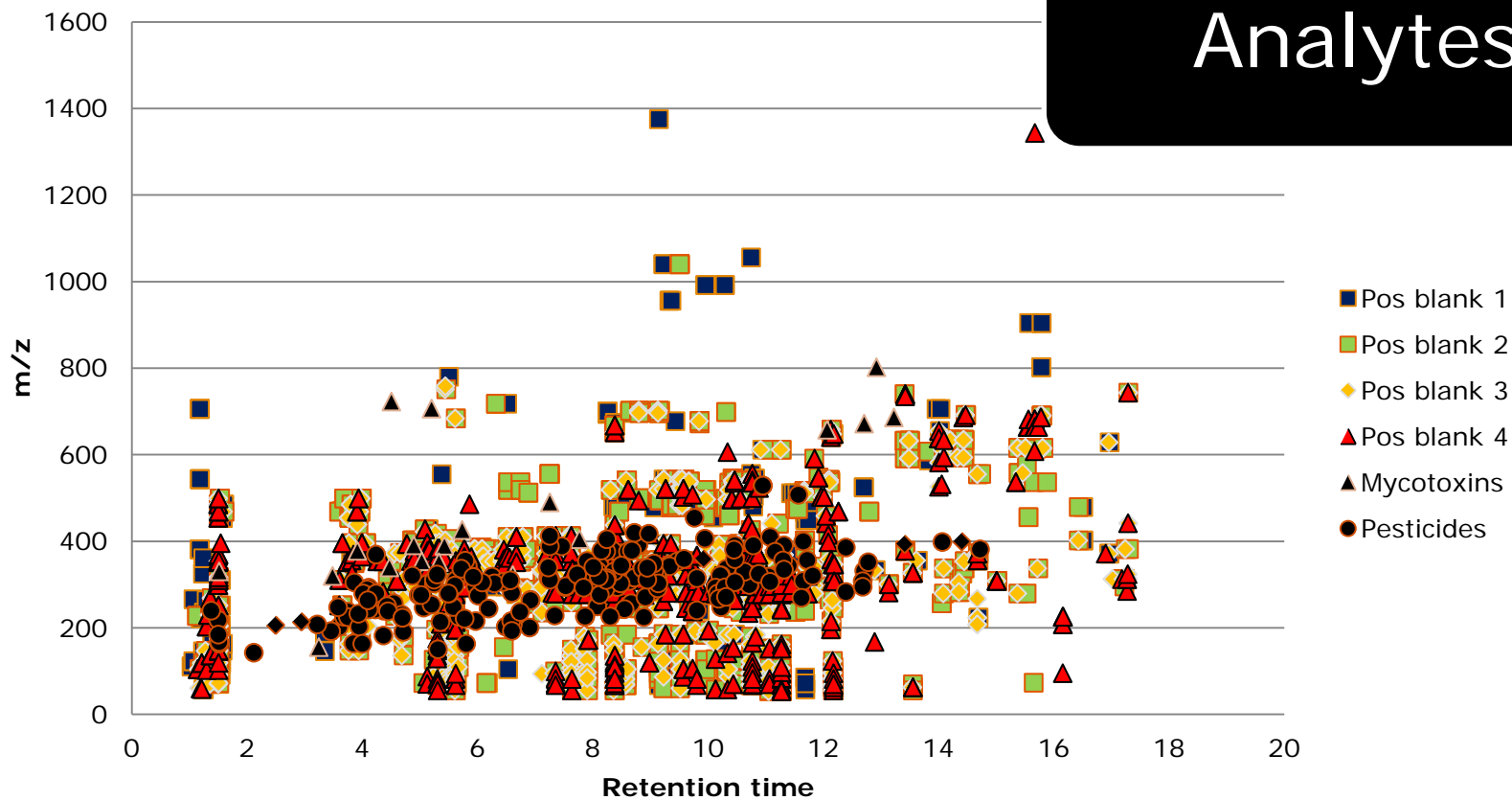
Positive mode

PSA clean up



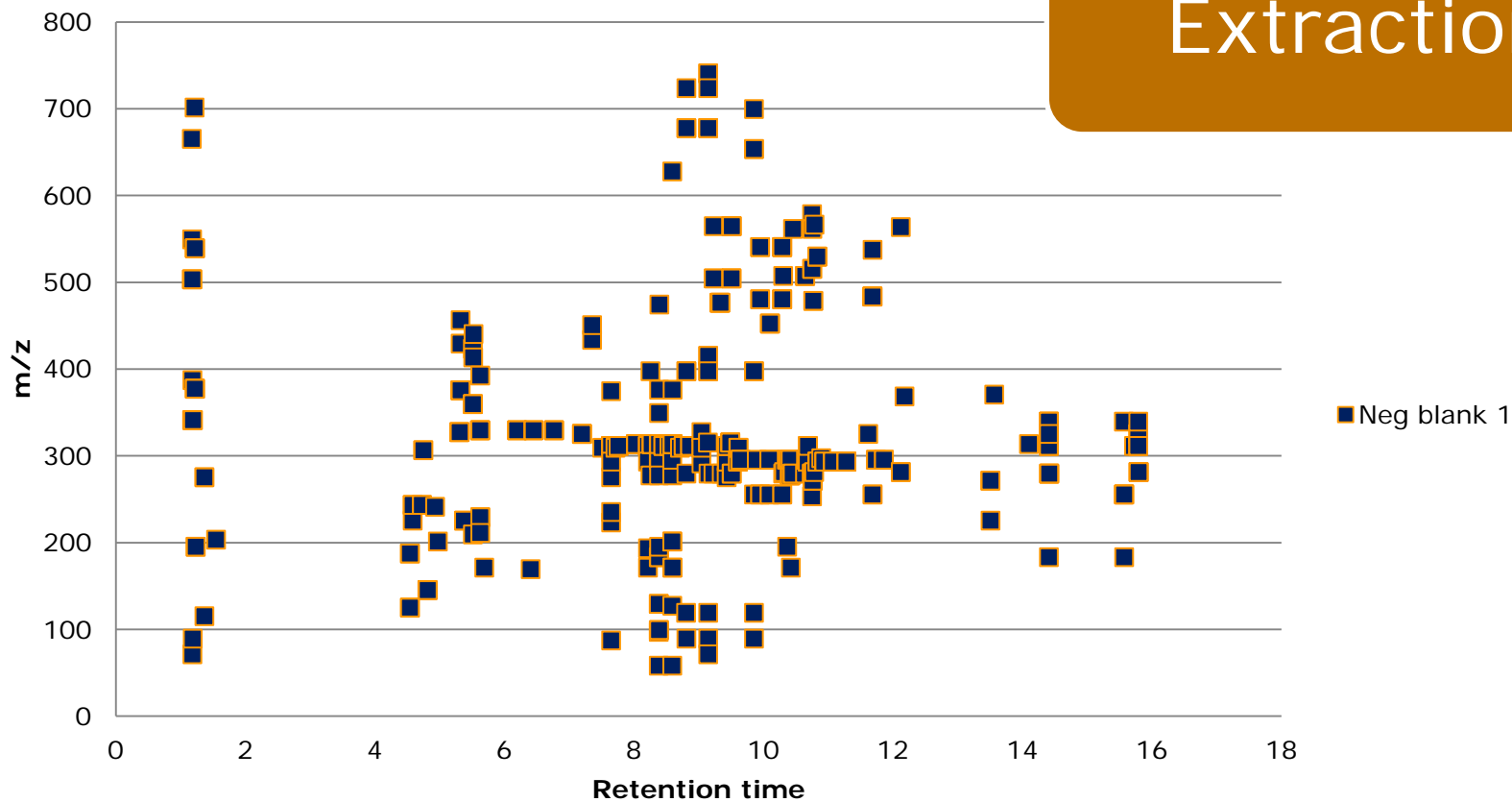
Positive mode

Analytes



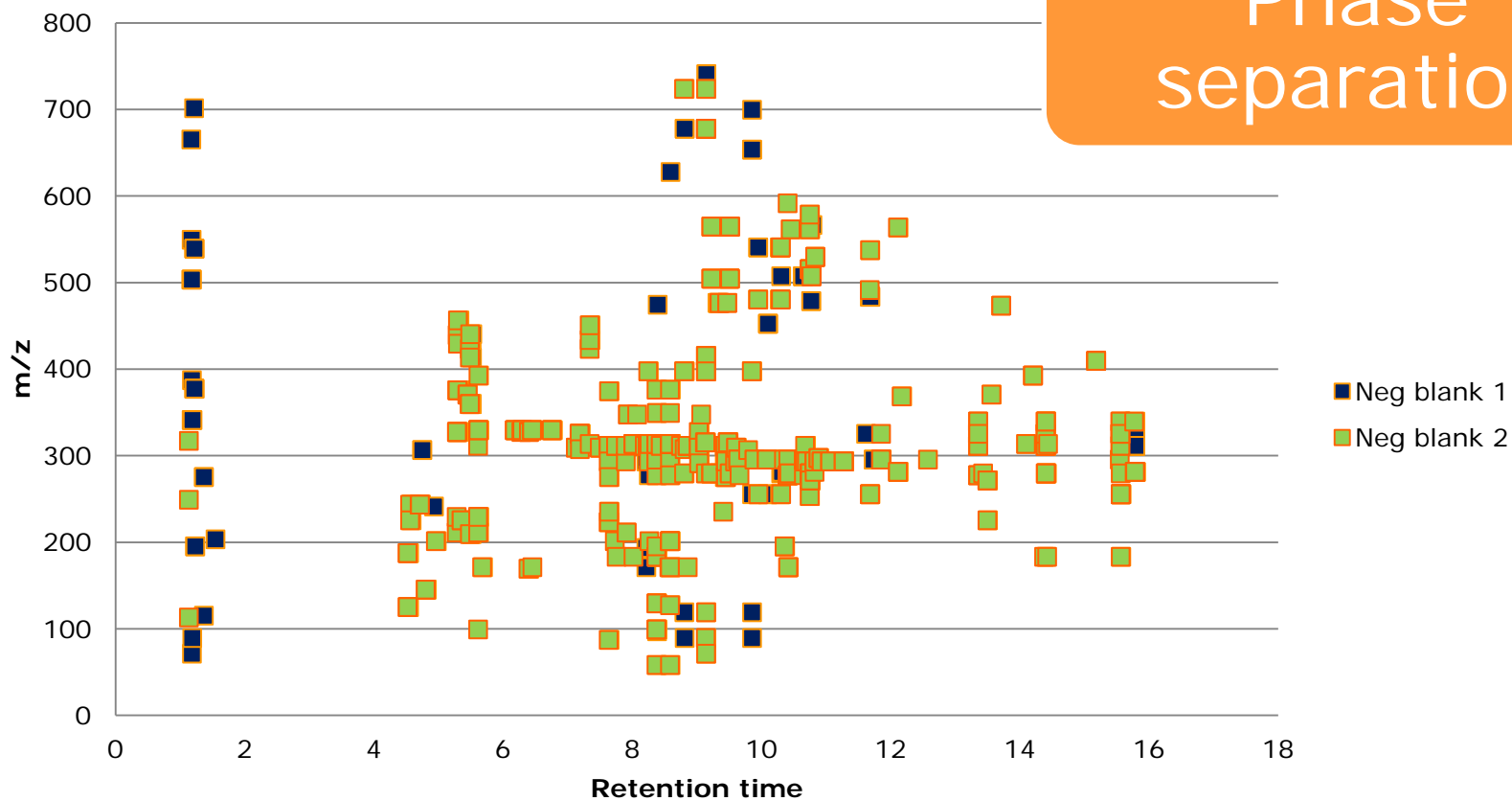
Negative mode

Extraction



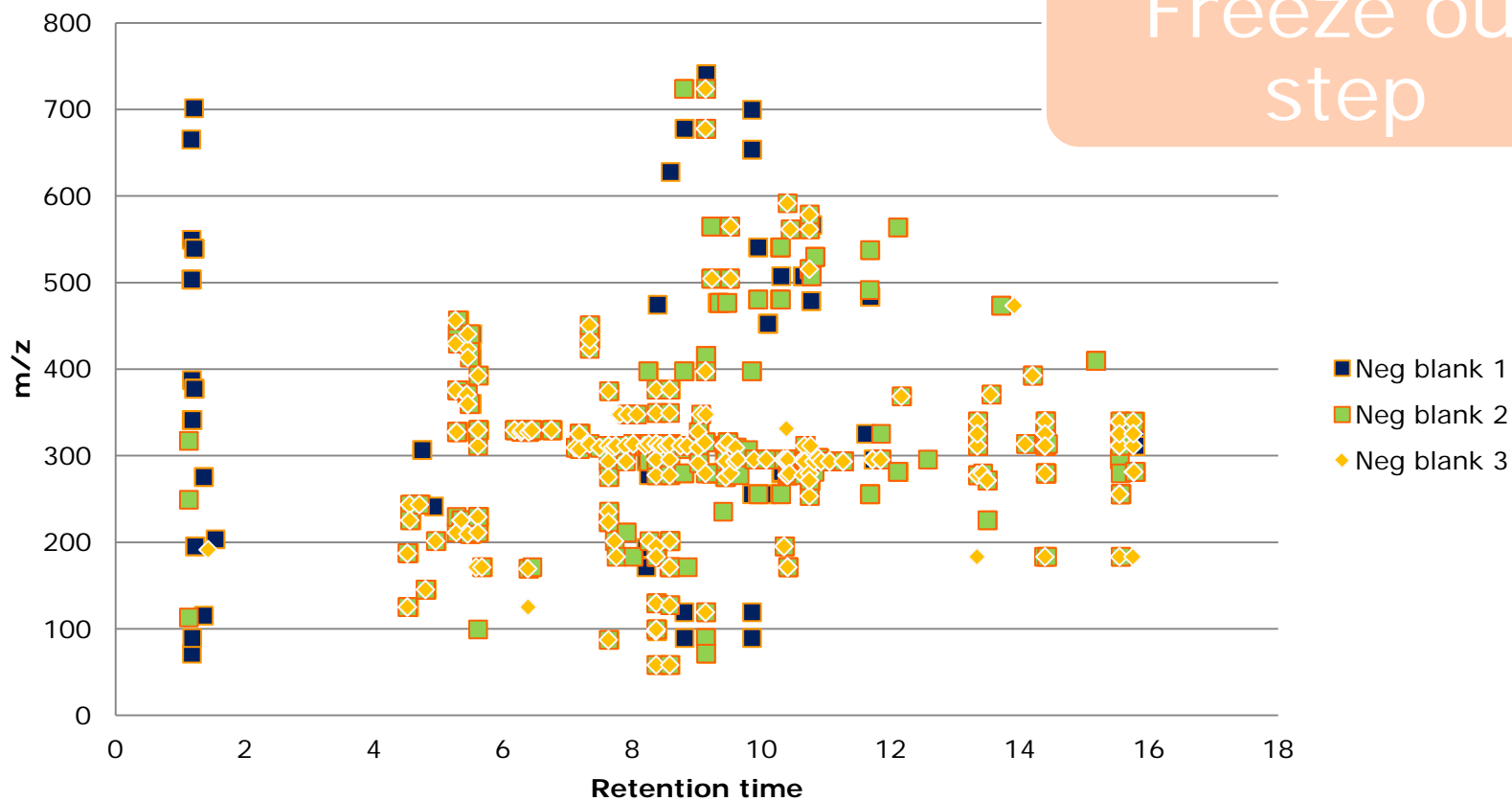
Negative mode

Phase
separation



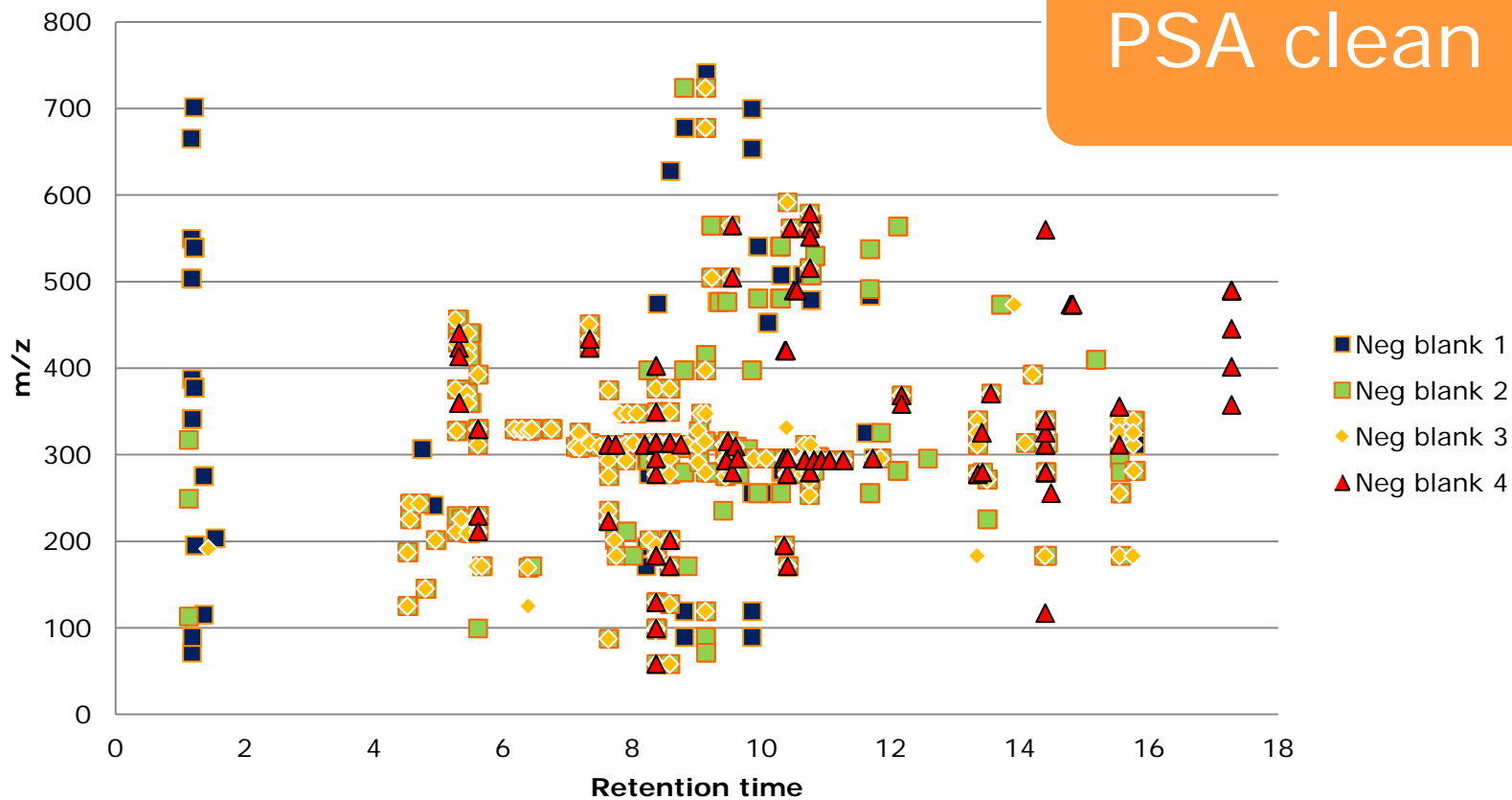
Negative mode

Freeze out
step



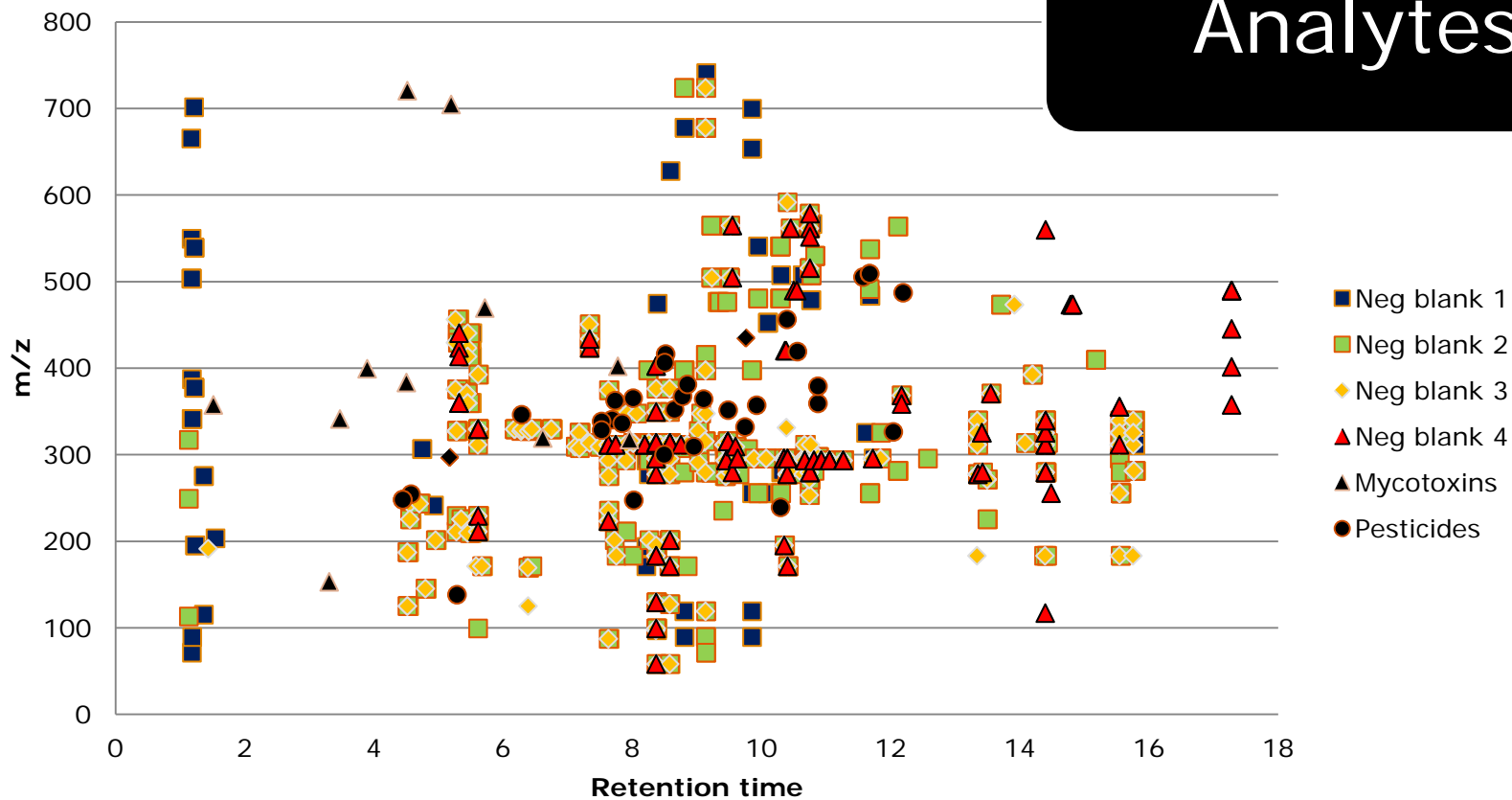
Negative mode

PSA clean up

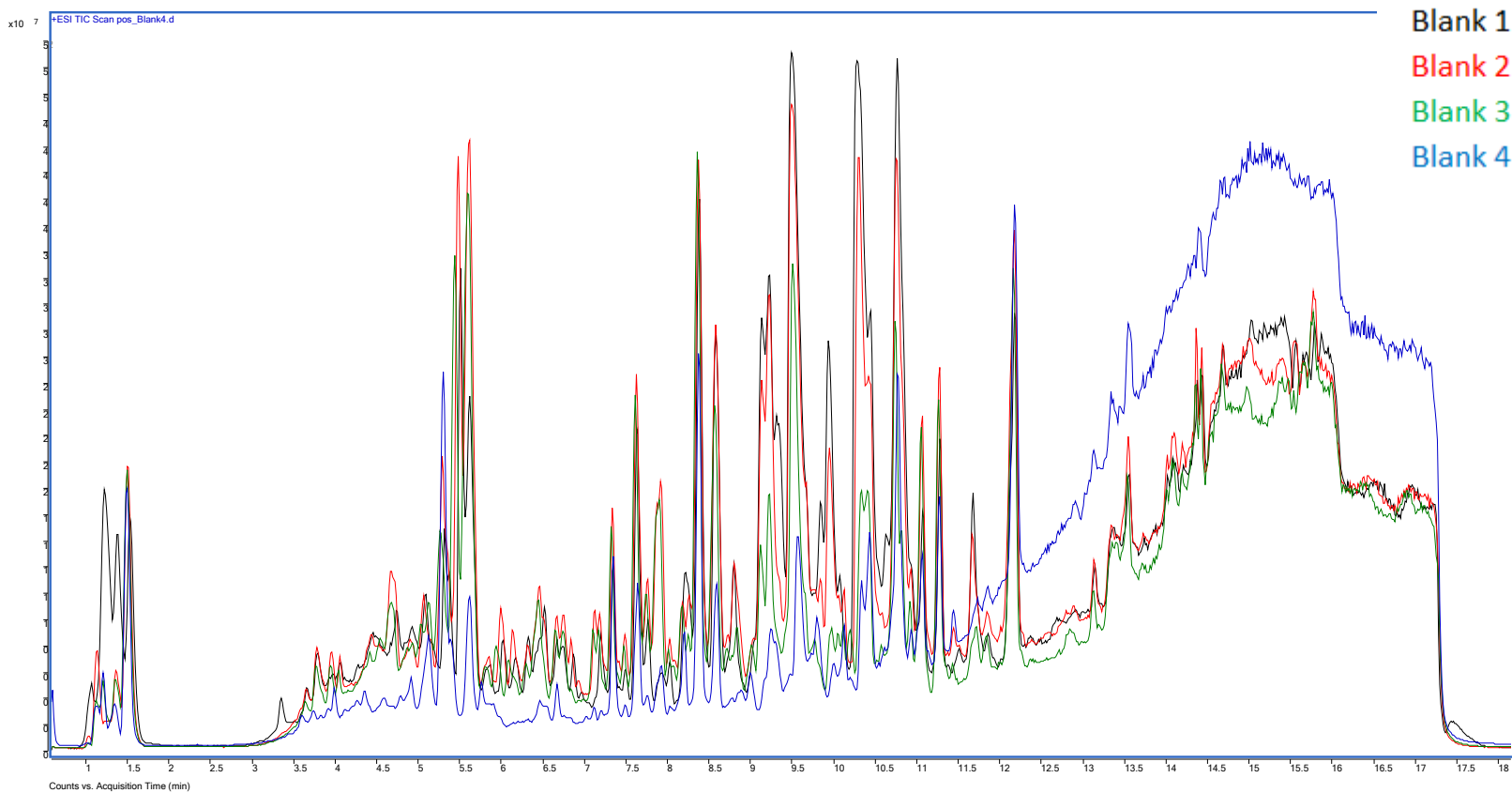


Negative mode

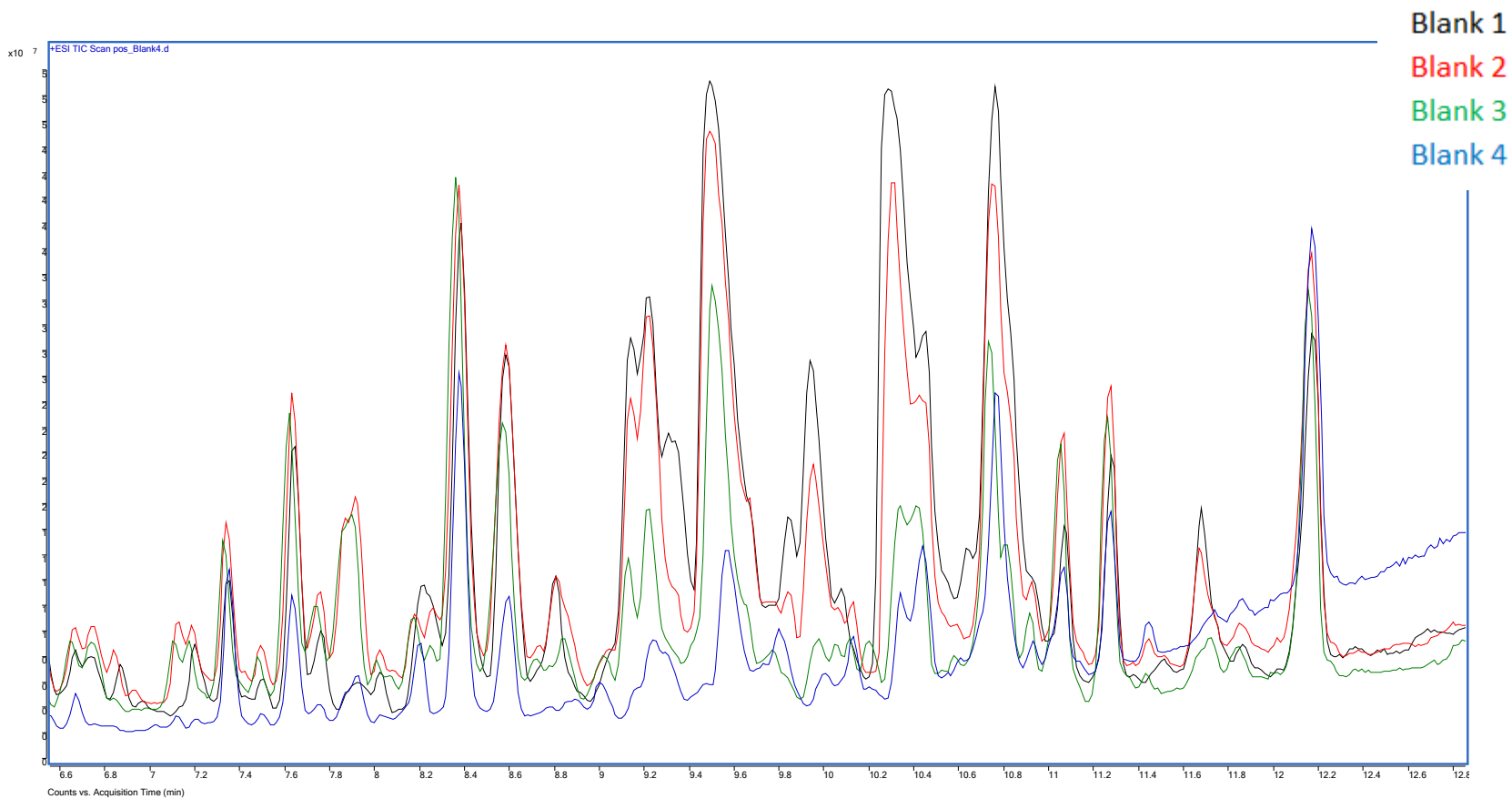
Analytes



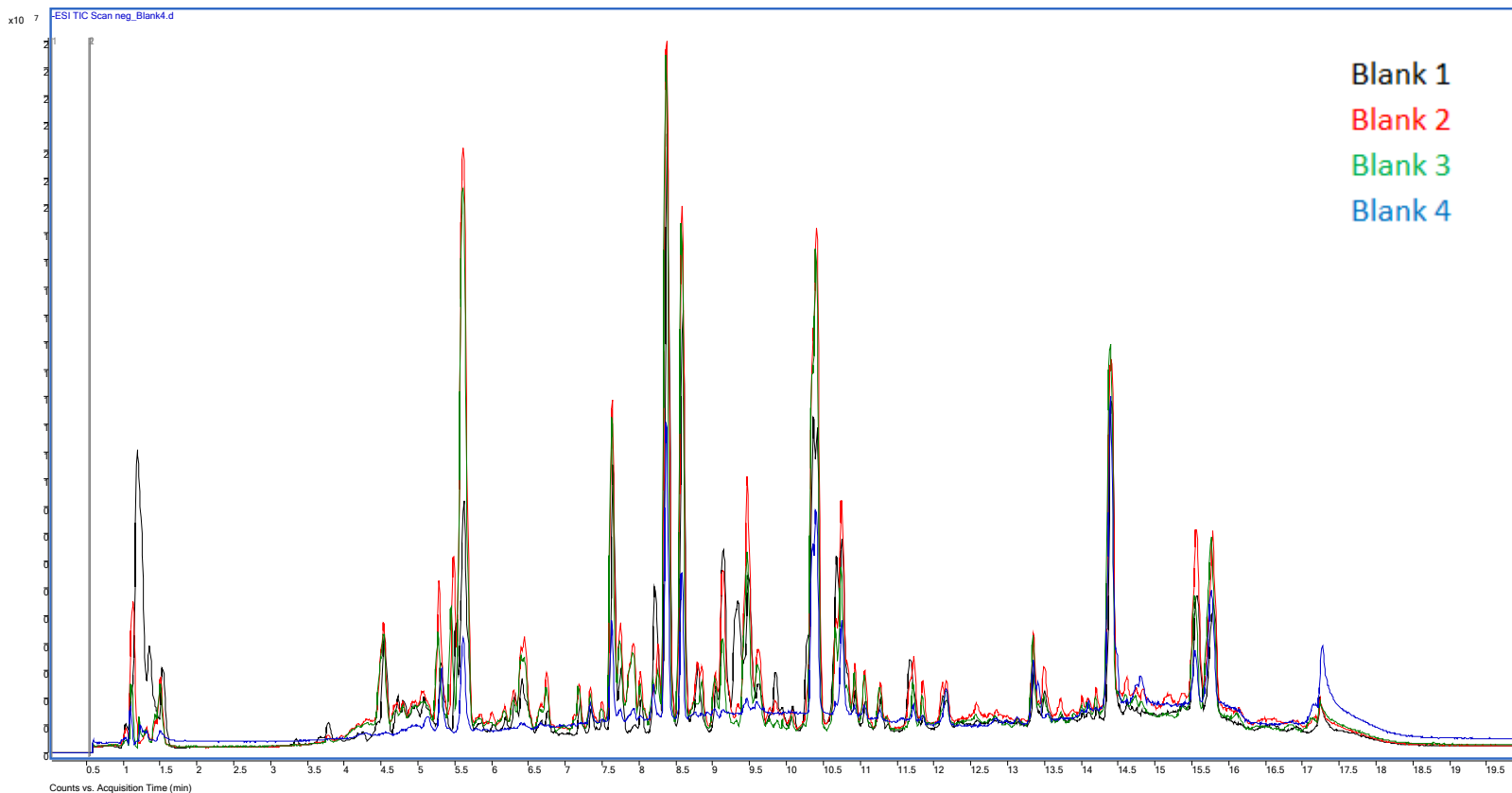
TIC af blank i positiv mode



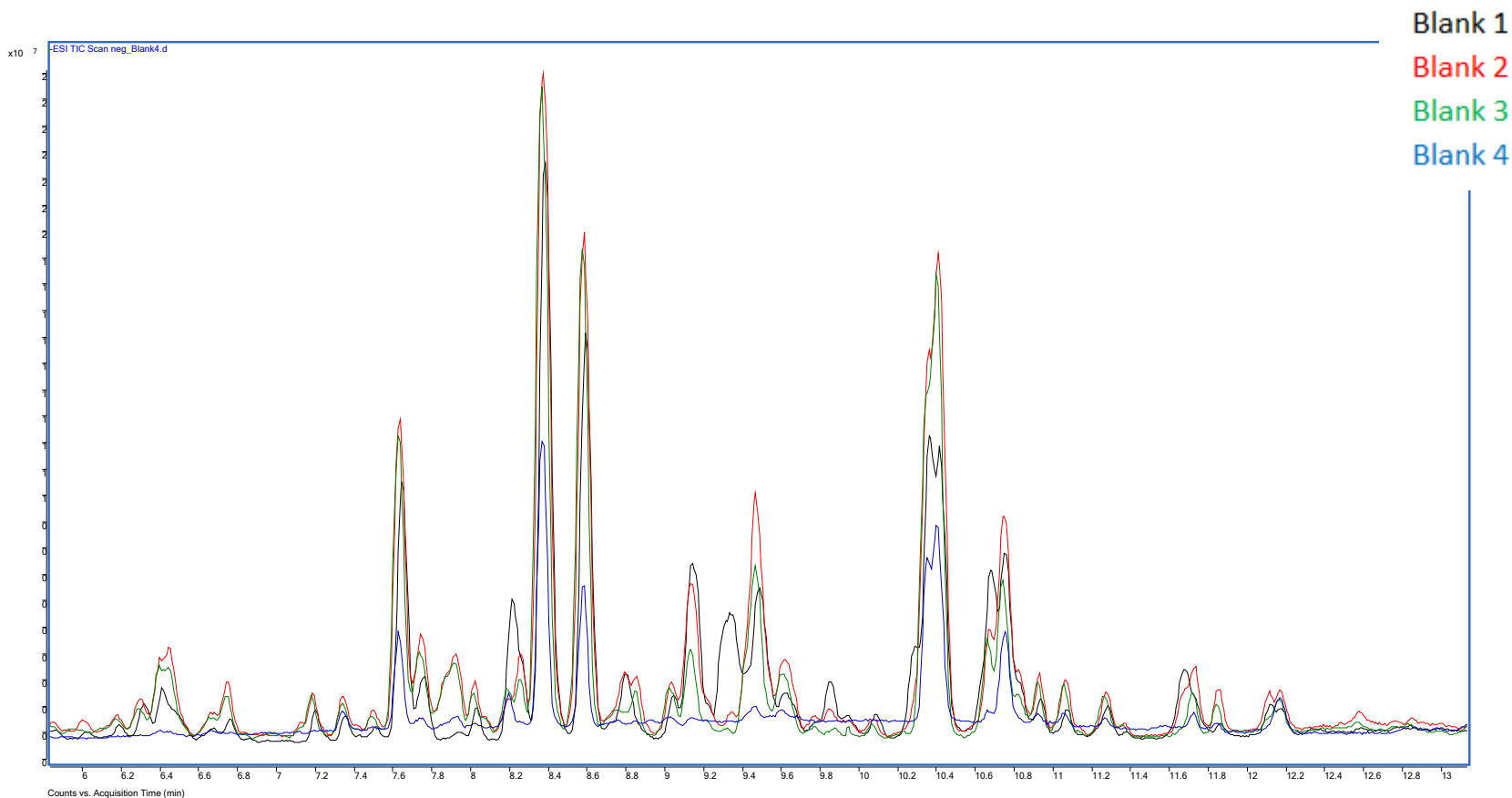
TIC af blank i positiv mode



TIC af blank i negativ mode

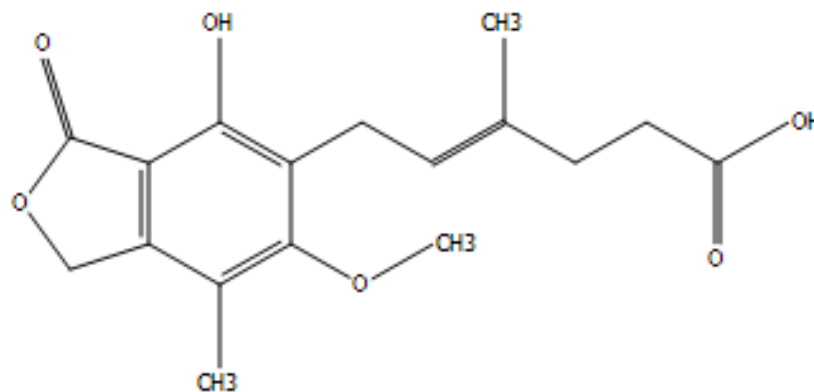


TIC af blank i negativ mode



Matrix effect & recovery – Mycophenol acid

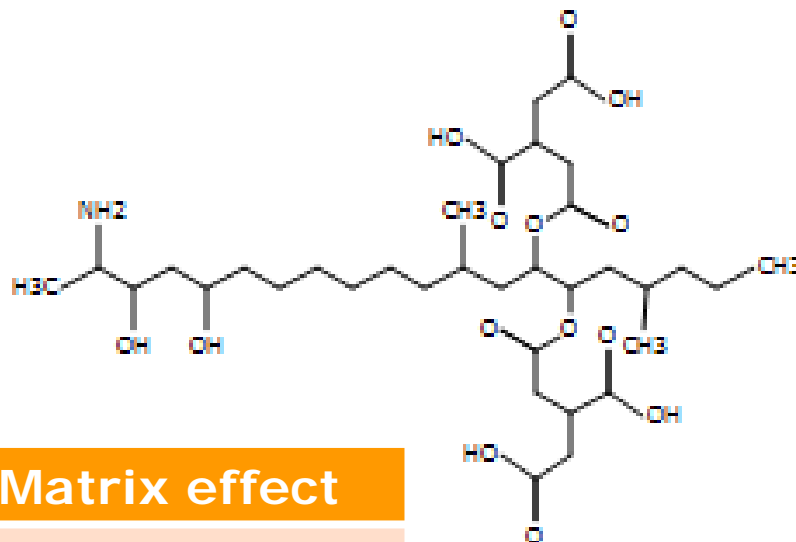
- Mycophenol acid
- 6.63 min
- Mass: 320.1260



	Recovery	Matrix effect
PSA	7	106
Freeze out	114	153
Phase separation	105	149
Extraction	123	159

Matrix effect & recovery – Fumonisin B2

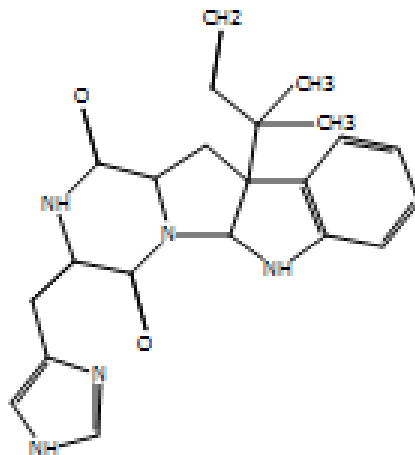
- Fumonisin B2
- 5.07 min
- Mass: 705.3936



	Recovery	Matrix effect
PSA	6	111
Freeze out	36	151
Phase separation	73	166
Extraction	76	153

Matrix effect & recovery – Roquefortin C

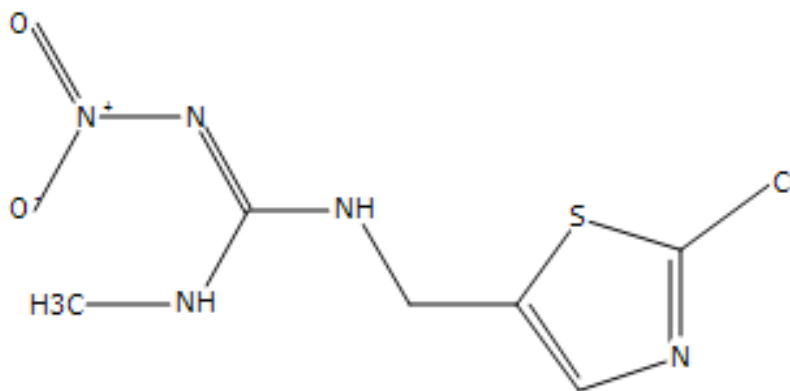
- Roquefortin C
- 5.15 min
- Mass: 389.1852



	Recovery	Matrix effect
PSA	92	75
Freeze out	92	41
Phase separation	101	38
Extraction	153	31

Matrix effect & recovery – Clothianidin

- Clothianidin
- 4.46 min
- Mass: 249.0087



	Recovery	Matrix effect
PSA	89	83
Freeze out	95	61
Phase separation	76	57
Extraction	153	32